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Research Article
Effect of Curcumin on Iron Toxicity and Bacterial Infection in Catfish (*Clarias gariepinus*)

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Abstract

Background and Objective: Iron is an essential element that involved in many vital physiological functions in fish, while excess iron concentration causes many toxic effects. Curcumin is a natural popular spice that used as a dietary supplementation and has iron chelating properties. This study was conducted to evaluate the effect of curcumin on iron toxicity in catfish (*Clarias gariepinus*). Also this study assess the antibacterial effect of curcumin against *Vibrio anguillarum* infection. **Materials and Methods:** *Clarias gariepinus* were orally exposed to low and high doses of curcumin (40, 80 mg kg^{-1} fish) for 3 weeks. Fish were then exposed to 25 mg L^{-1} of ferric chloride as a source of iron toxicity for another 3 weeks. Some hematological parameters (Total and differential white blood cells count, total red blood cells count, hemoglobin concentration and hematocrit %) and biochemical parameters (Serum ferritin, transferrin, ALT, AST, protein and albumin) were assessed before and after exposure to iron. Iron residues in gills, spleen, liver, kidney, abdominal fats, gonads and muscles were also determined. Moreover the determination of fish survivability after bacterial challenge with *Vibrio anguillarum* was recorded. **Results:** Iron caused decrease in total white blood cells count (WBCs), increase in ferritin level and elevation in liver function enzymes (ALT and AST). However, the pretreatment of fish with curcumin significantly increased WBCs, lymphocyte percentage, ferritin level and protein and albumin concentrations with significantly decreased transferrin, ALT and AST levels. Also there were significant decreases in iron concentration in serum, kidney, gonads and muscle in both low and high curcumin pretreated groups compared to Fe group. **Conclusion:** Results indicated a modulatory effect of curcumin against iron toxicity in catfish, also curcumin had an immune-stimulant effect against *Vibrio anguillarum* infection.

Key words: Catfish, curcumin, ferritin, iron toxicity, transferrin, *Vibrio anguillarum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Iron is one of the essential elements that involved in many metabolic processes for growth and development of organisms, while excess iron has many toxic effects and may leads to cellular and visceral damages[1]. Iron can be found naturally in water bodies from many geochemical and anthropogenic processes. Also, it can finds its way to the water through the pollution with industrial, sewage and agricultural drainage. Iron is highly oxidized and precipitates in the form of ferric hydroxide on the bottom of water bodies causing reduction in light penetration, decreasing productivity and food sources, stressing fish and reducing its growth[2,3].

The toxic effect of iron overload on many fish species have been studied; brook trout (*Salvelinus fontinalis*) were more vulnerable to infections after exposure to more than 6 mg L^{-1} suspended iron, while 12 mg L^{-1} retarded its growth and 50 mg L^{-1} reduced the egg viability[4]. Also, egg hatching, fry surviving and juvenile growth of fathead minnows (*Pimephales promelas*) were affected at exposure to 1.5 mg L^{-1} lime-neutralized suspended iron[2]. Moreover, an unfavorable effects on ova and spermatozoa of rainbow trout (*Salmo gairdneri*) fertilization were reported after exposure to 0.08 mg L^{-1} iron[5]. Both osmoregulation and sodium concentration in charr (*Salvelinus fontinalis*) were affected after exposed to 1.0 mg L^{-1} iron for 48 h[6]. Exposure of brown trout (*Salmo trutta*) to 2 mg L^{-1} of mixed ferric chloride and ferrous sulphate caused gill damage, increase in blood glucose and changed hematocrit values[7]. Also, over doses of iron may cause fish DNA damage[8].

Applications of medicinal plants in aquaculture is fast growing all over the world, their use in amelioration the toxic effects of some external pollutants on fish health was studied[9-11]. Curcumim is a wide board spice from family Zingiberaceae, used in many medicinal purposes; it has an antioxidant, anti-inflammatory, antibacterial and anti-cancer effects[12]. Few literatures concerning the effect of curcumin on fish are found, it has been used as a parasiticide[13], improving the growth performance[14], antibacterial and immunostimulant[15-17].

Now a days, the great challenge of medicinal plants is its using in fighting the pollution, this may be achieved through stimulate fish immunity, increase antioxidant capacity, minimize the pollutant side effects or by chelating the pollutant. Some studies were found that curcumin significantly reduced fish liver damage after toxicity with carbon tetrachloride[9] and aflatoxin[18]. Other studies pointed to the role of curcumin in reducing the toxic effect of iron overload in rats and mice[1,19,20], while there is no information about its role in reducing the iron toxicity in fish and its effect on free and stored iron forms. So, the aim of the present study is to evaluate the effect of low and high doses of curcumin oral administration on iron toxicity in catfish (*Clarias gariepinus*) and to determine its effect on some hematological and biochemical parameters. Also the study assess the antibacterial effect of curcumin against *Vibrio anguillarum* infection.

MATERIAL AND METHODS

This study was performed in the Hydrobiology Department, National Research Centre at the period from January-May, 2019.

Fish and Experimental design: Fish (*Clarias gariepinus*) were purchased from a local fish farm at Kafr El Sheikh Governorate, Egypt and transported alive to the lab to be acclimatized in aerated free-flowing freshwater aquaria (pH 6.7-6.9) for 2 weeks. Curcumin (Lobal Chemie) was firstly dissolved in dimethyl sulfoxide (DMSO) and further diluted by phosphate buffer saline (PBS, pH 7.2) to prepare a stock solution, stored at 4°C until used. A total of 270 fish were randomly distributed into 3 main groups, 90 fish per each (30×3 replicates). The 1st group was received a daily oral low dose of curcumin (40 mg kg^{-1} of fish), the 2nd one received high oral curcumin dose (80 mg kg^{-1} of fish), while the 3rd one; the control, received only DMSO oral dose. During acclimatization and experiment time, fish were fed daily with 2% of fish weight on an ordinary commercial diet. After 3 weeks of oral administration to curcumin, each group was subdivided into 3 subgroups, blood and tissue samples were taken from the 1st subgroups (pre exposure to iron), iron was added to the 2nd subgroups as $FeCl_3$ (M.wt. 162.21) in a concentration of 25 mg L^{-1}. After another 3 weeks of exposure to iron, blood and tissue samples were collected from the 2nd subgroups (post exposure to iron).

Bacterial challenge: After oral exposure to curcumin for 3 weeks, the 3rd subgroups of *Clarias gariepinus* were intra-peritoneal injected with 0.2 mL of (3×10^7 CFU) culture suspension of pathogenic *Vibrio anguillarum*[21]. This pathogenic bacterial strain was supplied by Bacteriology lab, Hydrobiology Department, National Research Centre, Egypt. Re-isolation of injected bacteria from all freshly dead fish specimens was carried out during the period of observation.

Sample collection: Blood samples were collected from the fish caudal vein by 3 mL syringe and divided into 2 parts, one of them was gently transferred to EDTA anticoagulant evacuated tubes for the hematological parameters. The other

part was left in room temperature for 2 h, then centrifuged at 3000 rpm for 15 min to get serum which kept in -20°C until be used in biochemical analysis. Tissue samples (gill, spleen, liver, kidney, abdominal fats, gonads and muscles) were collected and stored at -20°C until iron residues analysis.

Hematological analysis: Hematocrit (Hct %) was determined by centrifuging the blood sample in a microhematocrit centrifuge[22]. Hemoglobin concentration (Hb, g dL^{-1}) was determined by the cyanmethemoglobin method[23] using a spectrophotometer (Agilent Cary 100/300 Series UV-Vis, United State) at 540 nm. Red blood cell counts (RBCs) and white blood cell counts (WBCs) were calculated using an improved Neubauer haemocytometer under light microscope (400x magnification)[24]. Differential leukocyte counts were made after blood smears were air-dried and stained using Giemsa stain. Each leukocyte type was counted in 200 white cell, then its percentage was calculated regarding the total number of leukocytes. Erythrocyte indices for each sample were calculated; mean corpuscular volume (MCV), mean hemoglobin content (MCH) and mean hemoglobin concentration in the cell (MCHC) according to the following equations[25].

$$MCV = \frac{\text{Haematocrit}}{\text{Erythrocyte count}} \times 10$$

$$MCH = \frac{\text{Hemoglobin}}{\text{Erythrocyte count}} \times 10$$

$$MCHC = \frac{\text{Hemoglobin}}{\text{Haematocrit}} \times 100$$

Biochemical analysis: Serum total proteins[26], albumin[27], globulin; by subtracting albumin concentration from total protein concentration, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[28] were determined using Spectrum Diagnostics Kits, Egypt. The absorbance for each was read at its specific wavelength according to the kit instructions using spectrophotometer (AGILENT CARY 100/300 Series UV-Vis, UNITED STATE). Serum ferritin and transferrin (ng mL^{-1}) levels were achieved using colorimetric ELISA diagnostic kits, following the manufacturer's instructions and using an ELISA reader at 450nm (HANIL SCIENCE INDUSTRIAL UNION 32R).

Iron concentration in serum samples: Iron concentrations in serum samples were determined colorimetrically using iron determination kit, Bio-diagnostics. Absorbance was detected using spectrophotometer (Agilent Cary 100/300 Series UV-Vis, United State) at 535 nm.

Iron residues in different fish tissues: Different tissues (gill, spleen, liver, kidney, abdominal fats, gonads and muscles) were weighed, put in glass vials and digested in 4 mL of concentrated super pure nitric acid (Merck, Darmstadt, Germany). Then placed on a hot plate at 100°C. After complete digestion, the samples were cooled in room temperature, filtered and finally a distilled pure water was added to each sample to reach a volume of 10 mL. Metal concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer 3110, USA). The concentration of iron was expressed as mg kg^{-1} wet weight.

Statistical analysis: The obtained data were statistically analyzed using MSTAT-C program. All data are expressed as Means±standard error (SE) of the means.

Ethical approval: Ethical clearance and approval were followed according to the National Research Centre Ethics Committee.

RESULTS

Hematological parameters: The results for hematological parameters are presented in Table 1. Oral administration of *C. gariepinus* on curcumin for 3 weeks significantly increased the total leukocyte count (WBCs) ($p<0.05$) in both cur40 and cur80 groups (106.65 and 106.45×10^3, respectively) when compared to the control (85.93×10^3). However iron toxicity caused significant decrease in WBCs in group that not treated with curcumin (83.98×10^3) ($p<0.05$). Pre exposure to curcumin caused significant increase in WBCs in iron exposed groups, especially in high curcumin dose (cur80+Fe group). Also the lymphocyte percentage was increased in curcumin treated groups. On the other hand, total red blood cells count (RBCs), Hb% and HCT were not significantly affected in the different treatment groups ($p\geq0.05$).

Biochemical analysis: Oral administration of *C. gariepinus* on low and high doses of curcumin neither affected the storage form of iron (ferritin) nor transferrin ($p\geq0.05$) in groups that not exposed to iron toxicity (Table 2). Exposure to iron caused a significant increase in ferritin ($p<0.05$) (14.3 ng mL^{-1}) compared to the control group (12.7 ng mL^{-1}), while transferrin was insignificantly changed ($p\geq0.05$). Also the results showed a significant increase in ferritin level and a significant decrease in transferrin level ($p<0.05$) in groups of *C. gariepinus* that were pretreated with both curcumin doses (cur40+Fe and cur80+Fe) when compared to the Fe group (Table 2).

Table 1: Effect of low and high oral doses of curcumin on some hematological parameters of *Clarias gariepinus* fish pre and post exposure to iron toxicity

	Pre-exposure to $FeCl_3$			Post exposure to $FeCl_3$		
Parameters	Control	Cur40	Cur80	Fe	Cur40+Fe	Cur80+Fe
WBCs×10^3	85.93±6.3^{cd}	106.65±2.6^{a}	106.45±0.8^{a}	83.98±3.9^{ab}	95.43±4.4^{bc}	100.73±3.7^{d}
Lymphocytes (%)	77.43±5.1^{a}	85.75±2.1^{a}	87.63±1.6^{a}	84.50±2.2^{a}	84.25±2.3^{a}	84.25±1.0^{a}
Monocytes (%)	15.15±2.6^{a}	12.05±1.7^{a}	9.98±1.1^{a}	14.50±1.5^{a}	12.75±1.8^{a}	12.50±0.6^{a}
Neutrophils (%)	7.43±2.7^{a}	2.45±0.4^{b}	2.73±0.5^{b}	3.50±0.7^{ab}	3.00±0.5^{ab}	3.25±0.6^{ab}
RBCs×10^6	2.28±0.2^{a}	2.16±0.1^{a}	2.05±0.1^{a}	2.28±0.1^{a}	2.08±0.1^{a}	2.21±0.2^{a}
Hb (%)	12.98±1.5^{a}	13.38±0.4^{a}	9.83±0.6^{a}	13.50±0.5^{a}	12.78±0.5^{a}	11.78±0.6^{a}
HCT	25.10±3.9^{a}	24.48±1.1^{a}	22.90±1.5^{a}	23.80±1.3^{a}	21.81±1.2^{a}	21.68±1.2^{a}
MCV	111.30±6.5^{a}	109.80±2.7^{a}	112.80±4.9^{a}	104.40±1.9^{a}	105.20±1.7^{a}	102.30±1.2^{a}
MCH	60.05±1.5^{ab}	60.65±1.0^{ab}	61.70±1.0^{ab}	59.63±2.5^{ab}	61.93±1.4^{a}	55.60±0.5^{b}
MCHC	54.28±2.1^{a}	55.08±1.3^{a}	54.43±1.4^{a}	57.25±1.5^{a}	56.55±1.1^{a}	54.45±0.8^{a}
PLT×10^3	184.30±9.6^{c}	232.00±8.1^{a}	224.80±8.7^{ab}	230.80±26.3^{a}	254.80±18.6^{a}	193.30±39.2^{bc}

Data are represented as Mean±Standard error, means with the same letter within the same raw are not significantly different (p>0.05)

Table 2: Effect of low and high oral doses of curcumin on some biochemical parameters of *Clarias gariepinus* fish pre and post exposure to iron toxicity

	Pre-exposure to $FeCl_3$			Post-exposure to $FeCl_3$		
Parameters	Control	Cur40	Cur80	Fe	Cur40+Fe	Cur80+Fe
Ferritin	12.70±0.02^{c}	12.61±0.01^{c}	12.66±0.02^{c}	14.30±0.14^{b}	14.70±0.02^{a}	14.81±0.03^{a}
Transferrin	0.25±0.02^{ab}	0.22±0.005^{b}	0.27±0.001^{ab}	0.29±0.008^{a}	0.15±0.008^{c}	0.17±0.006^{c}
ALT	19.39±3.5^{b}	20.88±0.9^{b}	21.86±2.1^{b}	30.83±2.4^{a}	20.25±0.9^{b}	24.02±0.7^{b}
AST	47.29±3.7^{cd}	44.97±2.4^{d}	44.62±2.6^{d}	79.85±3.5^{a}	54.50±2.5^{bc}	56.75±2.5^{b}
Protein	2.72±0.16^{d}	3.83±0.13^{ab}	4.04±0.12^{a}	3.24±0.18^{c}	4.00±0.07^{a}	3.40±0.22^{bc}
Albumin	1.21±0.10^{c}	1.85±0.08^{ab}	2.00±0.19^{a}	1.62±0.15^{ab}	1.79±0.11^{ab}	1.51±0.08^{bc}
Globulin	1.52±0.20^{c}	1.98±0.17^{abc}	2.05±0.07^{ab}	1.63±0.24^{bc}	2.22±0.09^{a}	1.90±0.19^{abc}

Data are represented as Mean±Standard error, means with the same letter within the same raw are not significantly different (p>0.05)

Table 3: Iron concentration in serum (mg L^{-1}) and different organs (mg kg^{-1} wet weight) of *Clarias gariepinus* orally administrated to low and high doses of curcumin pre and post exposure to iron toxicity

	Pre-exposure to $FeCl_3$			Post-exposure to $FeCl_3$		
Parameters	Control	Cur40	Cur80	Fe	Cur40+Fe	Cur80+Fe
Serum	2.47±0.07^{b}	2.33±0.17^{b}	2.75±0.10^{b}	4.61±2.23^{a}	2.06±0.34^{b}	3.33±0.91^{ab}
Gill	34.33±1.83^{d}	34.87±2.63^{d}	34.74±2.81^{d}	43.51±1.97^{c}	80.45±2.69^{a}	50.14±1.28^{b}
Kidney	114.96±4.34^{b}	118.42±2.73^{b}	117.68±2.99^{b}	188.27±1.25^{a}	92.74±2.77^{c}	89.81±2.61^{c}
Spleen	96.23±4.04^{d}	95.63±3.67^{d}	98.07±2.46^{d}	126.85±2.87^{c}	303.21±0.56^{a}	243.20±2.04^{b}
Liver	129.45±2.89^{c}	130.98±7.91^{c}	128.28±1.74^{c}	179.36±2.50^{b}	228.15±2.11^{a}	121.60±2.50^{d}
Fat	4.90±0.93^{c}	4.39±1.18^{c}	4.29±1.05^{c}	9.68±1.45^{b}	11.84±0.54^{a}	1.35±0.32^{d}
Gonads	12.81±2.40^{b}	14.03±1.11^{b}	13.21±1.55^{b}	18.51±1.57^{a}	15.51±2.12^{b}	9.32±0.92^{c}
Muscle	11.11±1.75^{c}	11.30±2.05^{c}	11.85±2.42^{c}	30.94±2.73^{a}	13.20±1.10^{c}	16.37±1.39^{b}

Data are represented as Mean±Standard error, means with the same letter within the same raw are not significantly different (p>0.05)

Regarding liver function, ALT and AST enzymes showed insignificant changes in low and high curcumin treated groups compared to the control one, while in iron exposed group there were significant elevation in ALT and AST values ($p \leq 0.05$) (30.83 and 79.85 unit L^{-1}, respectively) compared to the control group (19.39 and 47.29 unit L^{-1}, respectively). It was worth to mention that curcumin modulated such toxic effect of iron and caused decreasing in ALT and AST in both cur40+Fe and cur80+Fe to be nearly equal to their levels in corresponding non-iron exposed groups (Table 2).

Moreover, curcumin significantly increased protein and albumin concentrations in *C. gariepinus* serum in both cur40 and cur80 groups compared to the control group ($p<0.05$). Also, in all iron exposed groups; either Fe group or curcumin pretreated groups (cur40+Fe and cur80+Fe), there were significant increase in protein and albumin levels ($p<0.05$) (Table 2).

Iron concentration in serum and different tissues: It was obvious that oral administration of *C. gariepinus* to low and high doses of curcumin didn't significantly affect the iron concentration ($p \geq 0.05$) in serum and other examined organs before exposure to iron toxicity (Table 3). While after exposure to iron toxicity, fish orally subjected to both low and high curcumin doses (cur40+Fe and cur80+Fe) showed significant decrease ($p<0.05$) in iron concentration in serum, kidney,

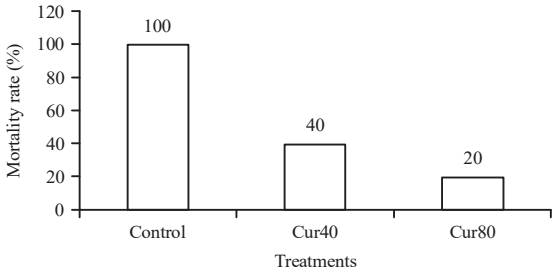

Fig. 1: Mortality rate of *Clarias gariepinus* injected with *Vibrio anguillarum* after oral administration to low and high doses of curcumin for 3 weeks

gonads and muscle compared to Fe group. This decrease in iron concentration was about one half the value in serum (2.06 and 4.61 mg L^{-1}), kidney (92.74 and 188.27 mg kg^{-1}) and in muscle (13.20 and 30.94 mg kg^{-1}) in cur40+Fe and Fe groups, respectively (Table 3). On the other hand, there was a significant increase in iron concentrations ($p<0.05$) in gill, spleen, liver and fat in fish pretreated with low curcumin dose (cur40+Fe) when compared with Fe group. This increase in iron concentration was about twice in gill (80.45 and 43.51 mg kg^{-1}) and in spleen (303.21 and 126.85 mg kg^{-1}) in cur40+Fe and Fe groups respectively (Table 3). Also, oral administration of *Clarias gariepinus* to high dose of curcumin (cur80+Fe) significantly increased ($p<0.05$) iron concentration in only gill and spleen organs after exposure to iron overload.

Bacterial challenge: Concerning the bacterial challenge test, fish survivability was the highest (80%) in the group fed with high curcumin concentration (cur80), while low curcumin group (cur40) showed 60% survivability compared to 100% mortality in control group (Fig. 1).

DISCUSSION

There is no doubt that iron is required for normal cell growth and proliferation. While, excess iron is harmful since it can help in formation of toxic reactive oxygen species (ROS) that harm living cells and retard its normal functions[29]. The application of medicinal plants to aquaculture is increasing rapidly in a trial to solve aquaculture problems, while more studies are needed to assess the effect of these plants on the various physiological and biochemical functions in fish to demonstrate its advantages and side effects.

Determination of different hematological parameters of fish gives an idea about the fish health and its physiological responses to any environmental stress[30]. Increase in WBCs and lymphocyte percentage in curcumine treated groups, in the present study, pointed to the role of curcumin in activation of the immune system. Similarly, Diab *et al.* and Elgendy *et al.* have proved that curcumin modulated the immune system in *Oreochromis niloticus*[15,16]. While the decrease in WBCs count in iron exposed group may be due to the increase in corticosteroids hormones that are secreted in fish exposed to any stress[31], such decrease in WBCs was modulated in pre curcumin exposed groups that may be explained by the chelating effect of curcumin and its ability to reduce free iron concentrations[1]. Also, curcumin was found to be safe in *Anabas testudineus* at doses of 0.5 and 1% and did not produce any hematological changes[32]. The present results were consistent to that since RBC, Hb% and HCT parameters were not significantly changed in the different treatment groups, indicating the safety of using curcumin to enhance the immunity of *C. gariepinus*.

Exposure to overload iron can achieve iron toxicity in which the levels of free iron in the body is increased. This free iron has the ability to donate and accept electrons and catalyze the conversion of H_2O_2 into free radicals that were responsible to the damage of many cellular structures and ultimately kill the cell. Controlling the intracellular iron levels can be regulated by ferritin and transferrin levels. Ferritin is an intracellular protein that stores Fe in non-toxic and soluble form to be released in need. Concentration of ferritin in serum indicates the total amount of iron stored in the body. Transferrin is an iron-binding glycoprotein which control the free-iron level, it is binding iron reversibly and deliver it from iron absorption centers to all tissues[33]. The present study showed that curcumin didn't affect ferritin and transferrin levels in fish under normal conditions. While in case of iron toxicity, curcumin increased ferritin level and decreased transferrin level. Such result may be returned to the chelating of excess iron by curcumin[1], in such a way by increasing its stored form and decreasing its free form. Moreover, these results were consistent with Pietsch *et al.*[34] who reported that some active compounds in natural plants can induce the transcription of ferritin mRNA.

Liver plays an important and vital role in iron homeostasis[35]. High concentration of iron causes damage in the metabolically active liver organ. Fe caused increased release of the transaminase enzymes (ALT and AST) into the blood circulation[36]. The results confirmed the toxic effect of iron and elevation in serum ALT and AST enzymes in Fe group. Fortunately, curcumin modulated such toxic effect of iron and significantly decreased ALT and AST values through chelating the excess free iron to not destroy cells. Some

previous studies have stated that curcumin had hepato-protective effects in many animals against different pollutants; such as carbon tetrachloride[37], endotoxin[38] and thioacetamide[39]. Other study revealed that the toxic effect of iron overload in rat has been reduced after oral administration to curcumin (100 mg kg^{-1})[19]. Also, the turmeric treated rats exhibited a protection against the toxic effect of iron overload on both hepatic and kidney functions, besides the reduction of iron levels in serum and tissues[1]. Curcumin is also reduce the intracellular production of reactive oxygen substances which mainly formed in metal toxicity and hence protect the cells from the oxidative damage[40], that may be attributed to its chelating effect on iron[1].

The present increase in protein and albumin in curcumin treated groups, may be explained by the immune-stimulant role of curcumin[16], also it denotes the well-functioning of hepatocytes in protein synthesis[41] and the antioxidant properties of curcumin[42]. The highest fish survivability in cur80 and cur40 confirmed the immunostimulant role of curcumin after bacterial infection. Such result was highly comparable with the high survivability of Nile tilapia fed on curcumin against *Vibrio alginolyticus*[16]. Curcumin antimicrobial effect may be attributed to its ability to suppress the proliferation of bacteria[43] and disrupt its cell division[44].

Bioaccumulation of heavy metals in fish depends on the absorbance and excretion rates[45]. The present increase in iron concentrations in gill, spleen, liver and fats and decrease in serum, gonads, kidney and muscle that were recorded in curcumin pretreated groups, ensure the protective role of curcumin against iron toxicity and chelation of excess free iron to be not stored in most vital organs, while it may trigger its excretion and bio-concentration in gill and liver. Previous studies stated increased pollutant concentration in gills because of its large surface area and it is the first organ to be in contact with water pollutants[46]. Liver is the main organ concerned with iron storage, it is responsible for bio-concentration and bio-accumulation of iron in different fish species, especially after exposure to iron overload[47]. Finally the present result recommend using of curcumin to reduce the iron overload toxic effect and to protect fish against bacterial infection.

CONCLUSION

The present results conclude that curcumin is completely safe on fish health, it didn't affect the hematological and biochemical main parameters and the iron concentration in different organs. Also it increased immunity of fish. While in case of exposure to iron toxicity, curcumin modulate toxic effect of iron overload on liver and chelate excess free iron.

SIGNIFICANCE STATEMENT

This study discovered the promising role of curcumin in modulating the iron toxicity in catfish (*Clarias gariepinus*). Also the study confirmed the antibacterial role of curcumin that can be beneficial for protecting fish against *Vibrio anguillarum* infection. So this study will help the researchers to use natural products to overcome pollution problems in aquaculture and reduce their toxic effects on fish health.

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